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Are humus forms, mesofauna and microflora in subalpine forest soils sensitive to thermal conditions?

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Abstract

This study focuses on the biological and morphological development of humus profiles in forested Italian Alpine soils as a function of *climate*. Humus form description, systematic investigation of microannelid communities and polyphasic biochemical fingerprinting of soil microbial communities (denaturing gradient gel electrophoresis – DGGE and phospholipid fatty acid analysis - PLFA) were performed to compare sites differing in mean annual temperature due to different altitude and exposure.

Although the soil biota showed complex responses, several differences in soil biological properties seem to be due to thermal differences. Although soil acidity also determines biological properties, it is not a state factor but rather influenced by them. The thickness of the organic layer and the acidification of the subjacent mineral horizon increased under cooler conditions (north-exposure; higher altitude), whereas the thickness of the A horizon inversely decreased. Species richness of microannelid assemblages was higher under warmer conditions (south-exposure; lower altitude) and the vertical distribution of microannelids shifted along the gradient to lower temperatures from predominant occurrence in the mineral soil to exclusive occurrence in the organic layer. Microbial biomass (total PLFA) was higher at the cooler sites; the prevalence of Gram-negative bacteria could be ascribed to their better adaptation to lower temperature, pH and nutrient contents. The $\delta^{13}C$ signatures of the PLFA markers suggested a lower decomposition rate at the cooler sites, resulting in a lower respiratory loss and an accumulation of weakly decomposed organic material. DGGE data supported the PLFA results. Both parameters reflected the expected thermal sequence.

This multidisciplinary case study provided indications of an association of *climate*, mesofauna and microbiota using the humus form as an overall link. More data are however needed and further investigations are encouraged.

Keywords: subalpine forest soils, humus forms, *climate*, microannelids, microbial community, microbial biomass

Introduction

Extreme environments such as high-altitude and high-latitude ecosystems are very sensitive to environmental changes (Diaz et al. 2003; Nemergut et al. 2005). Global warming is hypothesised to alter both above- and belowground processes affecting the soil ecosystem. This ecosystem is one of the most complex and dynamic biological systems on Earth and plays a major role in the biogeochemical cycles including weathering and nutrient dynamics (Young and Crawford 2004). Soil functionality depends, among others, on the functionality of soil biological communities (Nannipieri et al. 2003; Stark et al. 2008; Young et al. 1998).

Soil animals strongly affect microbial activity by altering the form of organic matter (OM). They fragment litter and mix it into the soil, which increases its susceptibility to microbial attack (Bardgett 2005). The relationship between microflora and saprophagous mesofauna allows hotspots and gradients of biological activity in a soil profile to be traced by the vertical distribution of mesofaunal actors such as enchytraeids (Galvan et al. 2008; Ponge 2003; Zanella et al. 2011a). At the species level enchytraeids are indicators of habitat conditions such as soil acidity or soil moisture. They are, therefore, often used in biological soil monitoring and assessment (Beylich and Graefe 2009; Graefe and Schmelz 1999; Jänsch et al. 2005).

The heterogeneity and discontinuity of the soil ecosystem render the assessment of the composition of microbial communities difficult (Nannipieri et al. 2003). Molecular techniques such as fingerprinting approaches based on nucleic acid- and phospholipid fatty acid revolutionised soil microbial ecology and have become routine methods for screening and monitoring complex soil microbial communities (e.g. Hirsch et al. 2010; Mannistö et al. 2007; Nakatsu 2007). Cellular biochemical constituents, such as lipids and nucleic acids, can be extracted directly from environmental samples without the need to store or culture microbes. Analysis of these constituents makes it possible to obtain biochemical fingerprints of communities and yields information about the taxonomy, functions, physiology, and abundance of community members (Green and Scow 2000). Climatic (and pedoclimatic) factors strongly influence the development of humus forms (Bonifacio et al. 2011; Ponge et al. 2011) which reflect the activity of different functional species groups of the soil fauna (e.g. epigeic, endogenic and anecic earthworms). The close relation between humus forms and the soil biota is based on the fact that humus forms result from the activity of soil organisms and at the same time act as habitat for them (Andreetta et al. 2011; Lalanne et al. 2008).

Due to the complexity of the soil system, the influence of *climate* on soil properties is not so easily detectable. Soil warming experiments can reveal information on the response of microbial communities and soil organic matter (SOM) with respect to direct higher temperatures. Many short- to long-term experiments in several ecosystems have been carried out so far. Some short-term experiments have shown that a rise in soil temperature increased the pool size of substrate C available for microbial respiration through a compositional and functional shift in microbial community

(e.g. Zogg et al. 1997). Longer-term studies have indicated that soil warming reduces microbial biomass and alters microbial community composition (e.g. Frey et al. 2008). Egli et al. (2010a,b) predicted that a warmer *climate* could intensify microbial activities in cold subalpine and alpine zones, which could cause consequent changes in humus chemistry and soil organic carbon content. Similarly, Hagedorn et al. (2008) state that elevated CO2 can accelerate the turnover of native SOM and may thus induce increasing losses of old C from thick organic layers of alpine soils. However, global *climate* change involves not only changes in air and soil temperatures, but also in soil moisture and vegetation. Therefore, the complex interactions of the various factors involved should be considered when assessing potential influences of future *climate* change (Budge et al. 2011). One possibility to study long-term effects of environmental factors (and in particular *climate*) on soil properties is to use sequences as defined by Jenny (1941, 1980). According to Jenny (1941), soils are understood to develop under the influence of the state-factors *parent material*, *climate*, *topography*, *biological activity* and *time*. In order to be quantitatively useful, Jenny (1941) suggested treating the state factors as independent variables, in the sense that field locales exist where the factors can be considered to vary independently. The ways in which a factor is considered to be independent is (1) if the range of variability of the factor is quite small and (2) if variation in the factor is large, it has only a negligible effect on the property studied.

In the present study we focused on the state factor *climate* and its influence on soil biological characteristics. Sites were chosen (according to the paradigm of Jenny, 1941) having the same topography, parent material, surface age and vegetation. The thermal conditions are the only environmental driver that differed between the sites. The soil organisms have been assessed in terms of diversity (evenness and richness) and vertical distribution of microfauna (microannelids) and microflora (bacteria, fungi) along the profiles. We hypothesised that i) thermal-related differences can be detected in the soil biota and ii) humus forms can be used as an indicator of soil biodiversity. According to the chosen sites (see below), we assumed that an altitude difference of 300 m should equalize the thermal effect of north vs. south exposure. The humus form is the seat of feed–back interactions, within the topsoil of a given local ecosystem, among roots, animals and associated biodegrader communities (Ehrenfeld et al. 2005; Zanella et al. 2011b). Changing the ecological frame (*climate*, *parent material*, *human pressure*, *history*), the system evolves consequently into new biocenoses characterized by adapted humus forms (Ponge 2003). Consequently, we assumed that the biological parameters and the humus forms trace back the thermal conditions of the chosen soil profiles. Furthermore, we hypothesised that the humus forms are the morphological link between SOM decay and soil biota.

Material and methods

Study sites, soil sampling and sample preparation

Experimental approach and study area

The soil profiles were selected from an existing soil cartography study and soil inventory (Sartori and Mancabelli 2009) and have been previously found to be typical and representative for the main parameters (altitudinal zones and exposure) affecting soil biotic and abiotic properties (Egli et al. 2009, 2010a,b). Geochemical characteristics of subalpine to alpine forest soils as a function of exposure and *climate* have been already accurately evaluated (Egli et al. 2007, 2009, 2010a,b). Due to the detailed biological and biochemical analyses (see below), only a very limited number of sites could be studied. Two pairs of soil profiles in the subalpine range in Val di Fassa (Southern Alps, Trento, Italy) were investigated. Each pair consisted of a north- and south-facing site. One pair (site Sorda) was located at about 1600 m asl and the other (site San Nicolò) at about 1900 m asl (Table 1).

Differences in surface temperature between north- and south-facing sites in Alpine areas is usually between 2-3°C. Combined together with a mean annual temperature gradient of 0.65°C/100 m (Sboarina and Cescatti 2004), then the following thermal sequence for the chosen sites is given (Table 1):

Sorda 1 (1620 m asl, south) > Sorda 4 (1640 m asl, north) ≥ San Nicolò 4 (1915 m asl, south) > San Nicolò 2 (1920 m asl, north).

The investigated soils have developed on basaltic latite (morainic material) and are Umbric (Sorda site) and Entic (San Nicolò) Podzols (IUSS Working Group WRB 2006). All sites have a natural forest (ecological forestry; Piceetum). Maximum precipitation occurs during the summer and autumn months. Using such an approach, a first database and insight into biotic and abiotic relationships can be gained. The sites do not differ with respect to the state factors (Jenny, 1941) except for *climate* (different mean annual temperatures and different exposures). Following this paradigm, differences between sites (and corresponding soil properties) should be largely attributed to the state factor *climate*.

Soil sampling

Special attention was given to ensure that the soils, according to their macromorphology, showed no sign of erosion or burial (undisturbed evolution). Sampling was done according to the morphology of the soils, as a soil horizon is a rather uniform compartment with typical chemical and mineralogical processes. Soils were sampled in July 2008. For physical-chemical analysis, 2 – 4 kg of soil material was collected per soil horizon from the 4 soil pits. Undisturbed soil samples down to the C horizon were taken. The soil samples were air-dried; large aggregates were gently broken by hand and sieved to <2 mm (fine earth). Following ISO 23611-3 (2007), the analysis of microannelid fauna was carried out using a split soil corer (diameter 5 cm) taking one soil sample per site to a depth of 30 – 45 cm, depending on the

thickness of the topsoil. The soil column was divided using a knife into subsamples of 5 cm depth intervals starting at the top of the organic layer. Subsamples were transported in plastic bags to the laboratory. For molecular analysis of soil microbial communities (PLFA-FAME; SSU rRNA gene fragment-PCR-DGGE) all the horizons of the opened soil profiles were vertically sampled in triplicate by using sterile Falcon tubes (50 ml), transported on ice to the laboratory and pooled together in order to obtain a composite sample of each horizon. Representative soil aliquots of each horizon/profile/site were sieved at 2 mm and stored at -20°C until DNA and PLFA extraction.

Humus forms

The description of the humus forms is based on two systems: European Humus Forms Reference Base (Zanella et al. 2011a) and the German system (KA5: Ad-hoc-AG-Boden 2005). The German name is given in brackets in the text. The horizon designation is according to the IUSS Working Group WRB (2006) and Soil Taxonomy (Soil Survey Staff 2010).

Microannelids

Depending on the profile and sequence of horizons, sampling was undertaken to a depth of 30-45 cm. Microannelid extraction from soil samples was performed over 48 h by a wet-funnel technique without heating (Dunger and Fiedler 1989; ISO 23611-3, 2007; van Vliet 2000). The extracted animals were counted and identified alive using both dissecting and light microscopy. Identification of the enchytraeid species was performed according to Schmelz and Collado (2010). Expert-knowledge based indicator values were used for assigning species to acidity indicator groups. Species with reaction values 1-3 according to Graefe and Schmelz (1999) were combined into ‘indicators of strong acidity’. Correspondingly, species with values 4-6 were combined into ‘indicators of moderate acidity’ and species with value 7 into ‘indicators of slight acidity’.

Soil microflora

Nucleic acid based genetic fingerprinting

Intracellular DNA (iDNA) was extracted from soil (0.5 g) by combining alkaline soil washings and mechanical chemical cell lysis, as described in Ascher et al. (2009a). Extracted iDNA was quali-quantitatively characterised by agarose gel electrophoresis and spectrophotometer measurements (Picodrop), respectively. Bacterial community 16S rRNA gene fragment-PCR was performed on 80 ng iDNA with the universal primers GC968f/UNI1401r (Nübel et al. 1996) as described in Ascher et al. (2010). The PCR products, 100 ng of 473 bp amplicons, were analysed by DGGE on

a 10% polyacrylamide gel (Acrylamide/Bisacrylamide 37.5:1) with an urea-formamide denaturant gradient of 45%-65% (100% denaturant contains 7 M urea and 40% formamide) at constant temperature (60°C) and voltage (100 V) for 16 hours (Ascher et al. 2010). Actinomycetes were additionally assessed as bacterial key stone species involved in humification processes by 16S rRNA gene fragment-PCR-DGGE using a half nested PCR approach (Heuer et al. 1997) on 40 ng iDNA with the primer set 243f/1401r (1st round PCR; 1175 bp) and GC968f/UNI1401r (2nd round PCR). The DGGE conditions were those described for bacteria. Fungal community 18S rRNA gene fragment-PCR-DGGE was performed using a nested PCR approach on 40 ng iDNA with the primer set NS1f/NS8r (1st round PCR) and the GC-clamped primer set EF4f/NS3GC (2nd round PCR with 2 µl of the first PCR product) (Ascher et al. 2009b). The final products, 100 ng of 500 bp amplicons, were analysed on a 10% polyacrylamide gel with a denaturant gradient of 30%-45% at constant temperature (58°C) and voltage (85 V) for 18 hours. DNA extractions, quantitative and qualitative analysis, PCR (MyCycler Thermocycler; Biorad), and DGGE (Ingeny PhorU system, Ingeny, Leiden, NL) were carried out in triplicates. DGGE gels were stained with SybrGreen I (1:10 000; FMC Bio Products, Rockland, ME USA; 2 h) prior to image analysis (GelDoc, Biorad).

Phospholipid fatty acid - fatty acid methyl ester analysis (PLFA-FAME)

Phospholipid fatty acids were extracted from soil (1 g) and derivatised to FAMEs using the method of Bligh and Dyer (1959) as adapted by White et al. (1979). The quantification and isotopic composition of individual FAMEs was determined using a GC Trace Ultra with combustion column attached via a GC Combustion III to a Delta V Advantage isotope ratio mass spectrometer (all Thermo Finnigan, Bremen, Germany). Samples (2 µl) were injected in splitless mode onto a J&W Scientific HP-5 column, 50 m length, ID 0.2 mm with a film thickness of 0.33 µm (Agilent Technologies Inc, Santa Clara, USA); running conditions were as described by Paterson et al. (2007). Quantification of individual PLFAs was achieved based on the combined area of the mass peaks m/z = 44, 45 and 46 after background subtraction and comparison with a 19:0 internal FAME standard added to each sample. The C isotope ratios were calculated with respect to a CO2 reference gas injected with every sample and traceable to International Atomic Energy Agency reference material NBS 19 TS-Limestone. The C isotope ratios of FAMEs were corrected for the C present in the methyl group added during derivatization using a mass balance equation. The precision of the δ13C measurement of individual PLFAs was obtained from the $\delta^{13}C$ values of the added 19:0 FAME internal standard which should remain constant; for all horizons this value was -32.80 ± 0.39 ‰ (mean ± SD, n = 16). The PLFAs used to indicate Gram-negative bacteria, Gram-positive bacteria, actinomycete and fungi were as described by Paterson et al. (2008).

Statistics

Similarities of microannelid structures as function of exposure, altitude, site and soil depth (vertical distribution) were assessed on the basis of presence-absence data of microannelid species (similarity index; Sørensen 1948) and reported in a trellis diagram. Principal component analysis (PCA) was performed on the relative abundance (percentage of total) of the total phospholipid fatty acid (PLFA) concentrations to assess for differences in microbial community structure. Thirty three individual PLFAs (variables) contributed to the total PLFA pool. Each sample was treated as an individual. No *a priori* knowledge of either the site or soil depth was assumed.

Similarities between microbial communities as a function of the assessed multiple parameters were assessed by the Dice similarity index based UPGAMA (unweighted pair group method using arithmetic averages) analysis of DGGE fingerprints using the Quantity One version 4.5.1. (Biorad). Pair-wise comparisons of prominent microbial community members/populations based on absence or presence of unique and shared DGGE bands were utilized and the resulting similarities (%) in microbial community structures among the studied soils were visualised by dendrograms.

Results

Soil mesofauna (microannelids) and humus forms

Sorda 1 south-facing (Fig. 1a, Table 2)

The extraction yielded a total of 145 animals which corresponds to an abundance of 74000 microannelids per square meter. Most animals (45%) were found in the topmost 5 cm which encompassed the entire organic layer (consisting of the Oi and Oe horizon) and the first 2 cm of the A horizon. With depth, the population density decreased at first rapidly and then more gradually along the A and BA horizon. Few worms were found yet in the lowest section reaching the Bs horizon. Thirteen different species were identified, all belonging to the family of Enchytraeidae. Species of the genus *Fridericia* (together 64%) and *Buchholzia appendiculata* (20%), a species inhabiting mainly the upper part of the humus profile, were the most dominant. *Buchholzia appendiculata* is a frequent species occurring at almost all sites. On the one hand the species is typical of the organic layer, on the other hand it is an indicator of slightly acid conditions. Grouping of species according to their tolerance of soil acidification reveals that 99% of the recorded animals are indicators of slightly acidic conditions. This is in agreement with the measured pH values ranging from 5.0 in the A horizon (Appendix A) to 6.3 in the Oe horizon (measured in field colorimetrically). The humus form (Table 1) is an intergrade type having features of a mull and a moder (KA5: F-Mull; ERB: Hemimoder). It is characterised by the absence of an Oa horizon, the presence of endogeic earthworms (*Octolasion lacteum*) and significant microannelid

activity in the A and BA horizon down to 45 cm depth. The vertical gradients of microannelid abundance and fungal and bacterial biomass in the mineral soil largely coincided (Fig. 1).

Sorda 4 north-facing (Fig. 1b, Table 2)

A total of 239 microannelids were extracted from the soil sample corresponding to 122000 animals per horizon. Their distribution was mainly restricted to the first 25 cm. The majority of animals (78%) were found in the upper 15 cm consisting of organic layers (Oi, Oe, Oa) and the AE horizon. Ten species were recorded, of which 9 belong to the family of Enchytraeidae and one to Polychaete (*Hrabeiella periglandulata*). Highly dominant were *Marionina clavata* (26%) and *Cognettia sphagnetorum* (24%), two species that are indicators of strongly acid conditions, whereas *Hrabeiella* (19%) is an indicator of moderate acidity. The former were found to live in the more acid horizons with high organic matter content including the AE, the latter in the less acid mineral soil underneath (Bs horizon). The humus form is a typical moder (KA5: Typic moder; ERB: Eumoder), because of the presence of an Oa horizon and the absence of endogeic earthworms in the mineral soil (no signs of endovermic activity were found). Typical for this soil is the gradual transition between the Oa and AE horizon which were difficult to separate morphologically. The vertical gradients of microannelid abundance and fungal and bacterial biomass show highest biological activities in the organic layer decreasing abruptly at the border of the very acid organic/mineral horizons. In contrast to Sorda 1, where the pH values are not very low, the pH($CaCl_2$) of the AE horizon at Sorda 4 is below 4.2 (aluminium buffer range), which may be toxic for some organisms.

San Nicolò 4 south-facing (Fig. 1c, Table 2)

Two hundred and thirty two microannelids were extracted from the soil sample corresponding to 118000. The majority of microannelids (61%) were found in the first 5 cm representing the organic layer and including a 3 cm thick Oa horizon. The population density decreased with depth, first rapidly and then more gradually along the AE and BA horizon to near zero in the Bs horizon. Thirteen different species were identified all belonging to the family Enchytraeidae. Dominant species in all soil depths were *Buchholzia appendiculata* (64%) and to a lesser extent *Henlea perpusilla* (12%) which occurred solely in the AE and BA horizon. The great majority (97%) of the extracted enchytraeids were indicators of slightly acidic soil conditions. This is in agreement with the measured pH ($CaCl_2$) ranging from 4.8 in the AE horizon to 5.1 in the BA horizon (Appendix A). By digging the soil pit, two specimens of *Aporrectodea rosea* and one specimen of *Octolasion lacteum* were found. Both species belong to the endogeic earthworms. The humus form is classified as Dysmoder (ERB) due to the presence of an Oa horizon, endogeic earthworms in the mineral soil (*Aporrectodea rosea* and *Octolasion lacteum*) and a significant but not dominant

microannelid activity in the AE and BA horizon down to 25 cm depth. According to Graefe and Beylich (2006) the humus form is an Amphi. The vertical gradients of microannelid abundance and bacterial biomass showed highest values in the organic layer, whereas fungal biomass was conspicuously lower there.

San Nicolò 2 north-facing (Fig. 1d, Table 2)

The extraction yielded a total of 80 microannelids corresponding to 41000. All animals were found in the organic layer, most of them (58%) in the first 5 cm consisting of Oi and Oe. No microannelid activity could be detected in the E horizon underlying the organic layer. 6 species were recorded, all belonging to the family of Enchytraeidae. The most dominant were *Henlea perpusilla* (36%) and *Buchholzia appendiculata* (35%). Both species are indicators of slight soil acidity. In contrast, *Cognettia sphagnetorum* (15%) is an indicator of strong acidity and occurred only in the first 5 cm. The pH of the organic layer (pH 5.0 measured in the Oi horizon) was unexpectedly high, but is in a full agreement with the species composition of microannelids, dominated by indicators of slight acidity. This interesting phenomenon could probably be due to some slope dynamics such as melt water run-off. It may explain the occurrence of mull-typical microannelids (*Buchholzia appendiculata*, *Henlea perpusilla*) in the Mormoder profile. The humus form is an aeromorphic mor (KA5: Mormoder; ERB: Humimor; Graefe and Beylich 2006: F-Moder) because of the zoogenic transformation of litter resulting in the sequence Oi-Oe-Oa, the sharp transition between Oi horizon and E horizon and the restriction of microannelid activity to the less acidic organic layer.

At all sites the vertical distribution of microannelids exhibited the highest density in the uppermost 5 cm of the profile. This section always included the organic layer (very thick at San Nicolò 2) or parts of it. Similar patterns emerge from the microbial data, thus providing evidence that the organic layer is the hotspot of biological activity in all four studied soil profiles (Fig. 1).

Soil microflora

Microbial biomass

The total PLFA concentrations, indicating the microbial biomass, decreased markedly with soil depth. This same trend was observed in the individual PLFA markers (Table 3). The decrease in bacterial PLFA with depth was relatively greater than that of the fungal PLFA as indicated by a decrease in the fungi/bacteria ratio (Table 3). The most abundant PLFA of soil was 16:0, a general biomass marker, followed respectively by 18:1ω7 indicative of Gram-negative bacteria, 18:1ω9 indicative of both fungi and bacteria and 18:2ω6,9 also indicative of fungi. Microbial biomass was found to be affected by all the evaluated parameters. The abundance (stocks) of PLFA was higher (in the topsoil as well

as over the whole soil profile; Table 3) at the north-facing site of Sorda compared to the south-facing site. In contrast to this, no such pronounced differences were observed at the site San Nicolò, suggesting a combined site and horizon effect masking the possible effect. The exposure effect becomes more evident when bacterial and fungal biomasses of the topsoils (south vs. north) were compared. With increasing altitude (and thus a cooler *climate*), the stocks (topsoil and whole soil profile) of total PLFA and fungi seem to increase. If we relate, however, total PLFA and fungi to the amount of org. C present in the soil, the situation completely changes. At the south facing sites, more total PLFA and fungi are present per gram C (Table 4). At all sites, however, the org. C-standardised amount of PLFA, fungi etc. decreases with soil depth indicating that the corresponding activity is in any case highest in the topsoil.

The yields of soil intracellular DNA (iDNA), representing an alternative/additional index of the microbial biomass (Ascher et al. 2009a, b), supported our obtained PLFA data (especially in the topsoil; Table 3; Fig. 2).

Microbial community structure

DGGE patterns were interpreted in terms of microbial community composition (diversity, dice similarity index based UPGAMA cluster analysis; absence-presence of bands) and phylotype richness (number of bands representing prominent populations of the communities) (Fig. 3). To facilitate the interpretation of the multiple factors (thermal conditions, soil depth) influencing microbial community structures, the main results are summarised in Table 5.

The evaluation of the exposure effect (north vs. south) showed the bacterial and fungal communities of both study sites (Sorda and San Nicolò) to be similar at the 48% and 15-20% level, respectively, suggesting a general effect of solar radiation on microbial community structures. The parameter soil depth generally discriminated top- from bottom-soil microbial communities, revealing fungi to be more affected with respect to bacteria. The parameter site generally selected microbial communities and was more pronounced for fungi (15%) than for bacteria (38%). The general lowest similarity and by inference the highest response to all the studied parameters was recorded for actinomycetes (5%).

A higher microbial richness was recorded for the San Nicolò site with respect to the Sorda site, suggesting a site effect (or thermal effect in general); this finding was in line with the overall PLFA abundances as well as individual PLFA markers.

By summing up all DGGE bands (phylotypes) of all studied soils (exposure/depth/site) (Fig. 3), the DGGE patterns seem to indicate more complex bacterial than fungal communities in terms of phylotype richness. This finding is supported by the respective PLFA data, indicative of bacterial and fungal biomass (Table 3) and DGGE cluster analyses of microbial community structures, showing higher similarities among bacteria (48%) than among fungi (15-20%) (Table 5).

Principal component analysis (PCA) was performed to assess the relative abundance and distribution of total phospholipid fatty acids (PLFAs) in all studied soils (Fig. 2). The space on the PCA graph of the relative abundance of PLFAs occupied by the top two horizons of the Sorda 4 (north-facing, 1640 m asl) and San Nicolò 4 (south-facing, 1915 m asl) profiles overlapped and differed from the space occupied by the top two horizons of both the Sorda 1 (south-facing, 1620 m asl) and San Nicolò 2 (north-facing, 1920 m asl) profiles (Fig. 2). This suggests that the microbial community structures of Sorda 4 and San Nicolò 4 – the sites with very similar thermal conditions at the soil surface - are most similar to each other. The fact that the space occupied by the subsoils differs from the topsoils provides additional evidence of a changing microbial community structure with depth. The latent vectors (loadings) of PCA score 1 indicated that the PLFAs 18:0 and 10-Me-18:0 had the greatest influence on the separation along this horizontal axis, with loadings of -0.31 and -0.29 respectively. 18:0 is possibly plant root derived and 10-Me-18:0 indicative of actinomycetes. In contrast, along the PCA score 2 axis, the loadings of -0.35 and 0.28 for 16:1ω11t and 18:1ω9 respectively, were most relevant. 18:1ω9 is indicative of fungi while 16:1ω11t does not represent a particular microbe group.

Consequently, the PCA data seem to trace back the thermal conditions (Table 1). From a thermal point of view, the following sequence (from warmer to cooler sites; with '>' as 'warmer than') is expected: Sorda 1 (1620 m asl, south) > Sorda 4 (1640 m asl, north) ≥ San Nicolò 4 (1915 m asl, south) > San Nicolò 2 (1920 m asl, north).

The low concentrations of PLFA found in the subsoils precluded an accurate C isotope determination of the less abundant PLFAs (Table 3). Of the four most abundant PLFAs, only 16:00, present in most microbes, showed a significant change in $\delta^{13}C$ with depth (i.e. enrichment in ^{13}C). In general, with increasing altitude and at north-facing sites (topsoils), the $\delta^{13}C$ of 16:00, 18:1ω7 and 18:1ω9 became slightly more negative. Consequently, the source of C utilised by the microbes is more enriched in the lighter ^{12}C indicating a less degraded OM fraction.

Discussion

The site effect, which contains a thermal signal due to the altitudinal difference of about 300 m, and to exposure (south vs. north), seems to exert an influence on mesofaunal and microbial communities, especially in the topsoil. The state factors *parent material*, *age*, *topography* and *organisms* (*vegetation*) do not vary among the sites. These factors can be, according to the paradigm of Jenny (1941, 1980), considered as constant or negligible. Differences in soil properties consequently should be mostly due to the state factor *climate* that is varying. Already earlier investigations in the North Italian Alps of the Trentino evidenced that *climate* exerts an influence on chemical and mineralogical soil properties (Egli et al. 2009, 2010a,b). Consequently, it was assumed that also soil biological properties must reflect climatic and thermal properties.

Mesofauna and humus forms

At Sorda, the humus form is changing between mull (ERB: Hemimoder; KA5: F-Mull) at the south-facing site and moder (ERB: Eumoder; KA5: Typic moder) at the north-facing site indicating a difference in humus forming processes. In mull, endogeic earthworms are mixing organic matter (OM) into the mineral soil and thus expanding the habitat for soil dwellers and reducing it for organic layer dwellers. At the south-facing site Sorda, this is evidenced by the deeper and the more even distribution of microannelids in the mineral soil, in contrast to the north-facing site where they are concentrated in large numbers in the upper part of the profile. At San Nicolò, the humus form varies between mull-moder (Graefe and Beylich 2006: Amphi; ERB: Dysmoder) at the south-facing and aeromorphic mor (KA5: Mormoder; ERB: Humimor) at the north-facing site. Compared to Sorda, the humus forms at San Nicolò indicate a shift to a slower decomposition of OM due to lower temperatures at the higher altitude. At the south-facing site the mixing activity of endogeic earthworms still occurred but not sufficiently to prevent the formation of an Oa (ERB: OH) horizon. According to ERB (Zanella et al. 2011a) such a system is defined as humus form amphi. Nonetheless, the sites with mull, amphi and moder humus forms showed high abundances of microannelids indicating a relatively high biological activity and a balanced OM turnover. The north-facing site at San Nicolò, however, underwent a longer phase of humus accumulation. The coincidence of a thick Oe (ERB: OF) horizon and the sparse number of microannelids indicates a low biological activity and may be an evidence that humus accumulation is still ongoing.

Although the number of observations is low, a tendency of thermal effects on the earthworms activity and microannelid composition was observed (Table 6). Our findings are in line with those of Salmon et al. (2008) who reported higher soil fauna activity at south- compared to north-facing sites. As known from previous investigations (summarised e.g. in Didden et al. 1997), the humus form as well as soil pH belong to the determining factors shaping the structure of microannelid communities. In the present study, the highest species number (13) was found at both south-facing sites. Furthermore, these sites showed highest similarity in species composition as expressed by the Sørensen index (Table 6). The overwhelming majority of species are indicators of slight acidity (Sorda 99%, San Nicolò 97%). Generally, indicators of slight acidity are associated with the humus forms mull and amphi that show no signs of strong acidification in the A horizon. A pH-value below 4.2 ($CaCl_2$) is considered being critical for the occurrence of these species (Graefe and Beylich 2003, 2006). The pH at both south-facing sites is above that value (Fig. 1). The north-facing sites of Sorda and San Nicolò exhibited lower species numbers of microannelids which is typical for humus forms (Moder, Mor) without mixing activity of earthworms (Beylich and Graefe 2009; Zanella et al. 2011a). At these sites soil acidification has progressed as far as to the aluminium buffer range. Soils with low OM content in that buffer range can be toxic for earthworms and microannelids (Graefe and Beylich 2003). This seems to be the case in the E

horizon at San Nicolò (north-facing). At Sorda (north-facing) the AE horizon was even more acidified, but rich in OM that prevents more toxic effects. The microannelids occurring in this horizon are almost exclusively indicators of strong acidity. If we consider the whole profile, half of the microannelids belong to this indicator group; the other half split into indicators of moderate (22%) and slight acidity (28%). Compared to the Sorda site, the proportion of indicator groups at San Nicolò is more shifted towards indicators of slight acidity (73%). This may be due to the higher pH values found in the organic layer.

The overall findings of the four studied sites demonstrate that exposure and altitude – and consequently the thermal conditions – seem to effect the microannelids population and the decomposer system. This demonstrates that the impact of exposure is probably stronger than previously assumed. The ecological similarity between the four studied sites is visualised (Fig. 4) based on the relative abundance of acidity indicator groups present in the microannelid assemblage.

Microbial biomass

Changes in microbial biomass due to thermal conditions (exposure, altitude) were found to be site-specific (Table 3). In contrast, Margesin et al. (2009) did not discover any significant differences in microbial communities in subalpine soils of the Austrian Central Alps. We measured a higher amount of Gram-negative than Gram-positive bacteria (Table 3). Higher amounts of both bacteria types in the topsoil were detected at the northern slope than at the southern slopes. The higher proportion of Gram-negative bacteria at north-facing sites and higher altitudes (cooler *climate*) can be ascribed to their better adaptation (compared to Gram-positive bacteria) to lower temperature, pH and nutrient contents (Margesin et al. 2009). The increased amount of these types of bacteria is, however, also due to the amount of org. C present. In fact, the concentration of these types of bacteria per gram C is at south-facing sites higher (Table 4). The amount of organic C in these environments is usually strongly related to *climate* (Egli et al. 2009) with a higher amount at north-facing and cooler sites. The highest amount of actinomycete biomass (abundance of 10-Me-18:0) was found in the organic-matter-rich horizons of all the studied profiles, supporting their role in humification processes (OM turnover). Actinomycetes (Gram-positive bacteria) are important members of the forest floor decomposer community and contribute to humification processes in natural soils along with white-rot fungi (Trigo and Ball 1994). The actinomycete abundance correlated with the amount of OM (org C and N; C stab and C lab) and pH (Appendix A). This is in agreement with findings of Jayasinghe and Parkinson (2008). High actinomycete abundance does, however, not necessarily indicate a high biodegradability of OM in the soil. The more intense acidification at north-facing alpine soils, the cooler *climate* and the higher podzolisation are suggested to be responsible for lower humification rates as indicated by the higher C/N ratios of the SOM and a higher amount of labile OM (Appendix A; Egli et al. 2009).

Microbial community structures

A generic view on microbial communities inhabiting the soil profiles indicates similarly strong thermal (due to exposure) effects on actinomycetes (5% similarity between south- and north-facing) > fungi (15 - 20%) > bacteria (48%; Table 5) for both sites (Sorda, San Nicolò). The evaluation of the site effect suggested a selective pressure on actinomycetes (5%) > fungi (15%) > bacteria (38%; Table 5). Furthermore, soil depth affected soil microbial community structures as reported by others (Douterelo et al. 2010; Fierer et al. 2003). This phenomenon can be ascribed to the fact that soil horizons are uniform compartments with typical physical-chemical and mineralogical characteristics (Egli et al. 2009, 2010a,b). In contrast to Margesin et al. (2009), our DGGE data also indicated a thermal effect on microbial community structures with generally lower similarities in fungal (15 - 20%) with respect to bacterial communities (48%) (Table 5). Although the acidity of the soils was not very pronounced due to the latitic and base-cation-rich parent material, a tendency to lower pH values at cooler sites seems to exist. The pH is among the most important factors influencing soil microbial structures (Mannistö et al. 2007).

The generally stronger response (lower similarities; DGGE) of actinomycete communities (5%) than of bacterial communities (48%) (Table 5, Fig. 3) to all evaluated parameters suggests that the actinomycete communities are a sensitive key population, capable of most accurately indicating shifts in community composition induced by environmental changes (Heuer et al. 1997). Due to their role in C degradation/humification processes, actinomycetes are strongly related to soil properties such as OM and pH. PLFA data (10-Me-18:0) supported the sensitivity of actinomycetes to environmental gradients as they mainly contributed to the separation along the horizontal axis (PCA score 1, Fig. 2).

The analysis of relative abundance and distribution of total PLFAs in all studied soils revealed the highest similarity of microbial communities between the top soil communities of the north-facing Sorda 4 (1640 m asl) site and the south-facing San Nicolò 4 site (1915 m asl). This fact corresponds to the expected thermal sequence (Table 1). This particular phenomenon was supported in part by the DGGE data; similarities of bacterial and fungal communities were found at a 48% and 39% level, respectively (Fig. 3). Furthermore, distinct differences in DGGE patterns are discernible for bacteria at the community level and are even more expressed at the group level (actinomycete patterns), suggesting thermal effects in terms of exposure (north vs. south) and altitude effects (Fig. 3). Lower temperatures, a higher soil humidity and more acidic conditions at the cooler sites stimulate, on the one hand, weathering processes and, on the other, affect the soil organisms (Egli et al. 2007, 2009, 2010a,b).

The most abundant individual PLFAs markers in all studied soil profiles were 16:0 (most microorganisms), 18:1ω7 (Gram-negative bacteria), 18:1ω9 (fungi and bacteria) and 18:2ω6,9 (fungi). The average ^{13}C enrichment of the 16:0 from the top to the bottom of the profile of 1.5 ‰ is consistent with previously observed ^{13}C enrichment of SOM with

depth (Agnelli et al. 2007; Boström et al. 2007; Risk et al. 2009). The more depleted $\delta^{13}C$ values of 18:2ω6,9 compared to the other PLFAs (Table 3) may reflect its mycorrhizal origin. As such, it would have obtained most of its carbon from host plants rather than direct assimilation of soil carbon. The differences in $\delta^{13}C$ values of 16:00, 18:1ω7 and 18:1ω9 must most probably be attributed to respiratory loss of ^{12}C-enriched CO_2.

Due to a lower microbial activity at the cooler sites, the average nutrient source for these microbes should be slightly enriched in ^{12}C (more less-degraded OM accumulates). Lower $\delta^{13}C$ values are indicative of less-decomposed OM and also a cooler *climate*, due to retarded decomposition processes. This agrees with the observations of Ning et al. (2006). In fact, less or weakly decomposed OM is encountered in soils at cooler sites (usually at higher altitudes or north-facing sites; see Egli et al. 2010a,b).

Conclusions

We obtained indications that thermal conditions (due to differences in altitude and exposure) – and consequently the state factor *climate* – seem to influence biotic soil characteristics (mesofauna and microflora) and related macromorphological properties (humus forms). Although soil acidity or nutrient availability are certainly important variables that also regulate biological properties, they are finally determined by the state factors.

To our knowledge this is one of the first studies that attempts to link (micro)biological factors to macromorphological soil properties and thermal conditions. Our preliminary, multidisciplinary results show that humus forms seem to be a good indicator for the soil biota (micro- and macro-biology). A larger dataset is, however, missing and additional investigations are necessary. High-altitude soils are predicted to experience a rapid warming in the future with distinct consequences for SOM quality, quantity (Egli et al. 2010a,b) or soil organisms (Budge et al. 2011). It is consequently of utmost interest to know which changes may occur with respect to soil biology and which might be key parameters for a monitoring and/or spatial extrapolation.

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References

Agnelli A, Ascher J, Corti G, Ceccherini MT, Pietramellara G, Nannipieri P (2007) Purification and isotopic signatures ($\delta^{13}C$, $\delta^{15}N$, $\Delta^{14}C$) of soil extracellular DNA. Biol Fertil Soils 44:353–361

Ad-hoc-AG-Boden (2005) Bodenkundliche Kartieranleitung – 5 (KA5). Auflage. Hannover, Germany

Andreetta A, Macci C, Ceccherini MT, Cecchini G, Masciandaro G, Pietramellara G, Carnicelli S (2011) Microbial dynamics in Mediterranean Moder humus. Biol Fertil Soils. DOI 10.1007/s00374-011-0622-9

Ascher J, Ceccherini MT, Chroňáková A, Jirout J, Borgogni F, Elhottová D, Šimek M, Pietramellara G (2010) Evaluation of the denaturing gradient gel electrophoresis (DGGE) - apparatus as a parameter influencing soil microbial community fingerprinting. World J Microb Biot 26:1721-1726

Ascher J, Ceccherini MT, Landi L, Mench M, Pietramellara G, Nannipieri P, Renella G (2009b) Composition, biomass and activity of microflora, and leaf yields and foliar elemental concentrations of lettuce, after in situ stabilization of an arsenic-contaminated soil. Appl Soil Ecol 41:351-359

Ascher J, Ceccherini MT, Pantani OL, Agnelli A, Borgogni F, Guerri G, Nannipieri P, Pietramellara G (2009a) Sequential extraction and genetic fingerprinting of a forest soil metagenome. Appl Soil Ecol 42:176-181

Bardgett RD (2005) The biology of soil: A community and ecosystem approach. Oxford University Press, Oxford, UK

Bastida F, Barberà GG, Garcìa C, Hernàndez T (2008) Influence of orientation, vegetation and season on soil microbial and biochemical characteristics under semiarid conditions. Appl Soil Ecol 38:62-70

Beylich A, Graefe U (2009) Investigations of annelids at soil monitoring sites in Northern Germany: reference ranges and time-series data. Soil Organisms 81:175-196

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Physiol Pharm 37:911-917

Bonifacio E, Falsone G, Petrillo M (2011) Humus forms, organic matter stocks and carbon fractions in forest soils of northwestern Italy. Biol Fertil Soils 47:555-566

Boström B, Comstedt D, Ekblad A (2007) Isotope fractionation and 13C enrichment in soil profiles during the decomposition of soil organic matter. Oecologia 153:89-98

Diaz HF, Grosjean M, Graumlich L (2003) Climate variability and change in high elevation regions: past, present and future. Climatic Change 59:1-4

Didden WAM, Fründ HC, Graefe U (1997) Enchytraeids. In: Benckiser G (ed) Fauna in Soil Ecosystems. Recycling Processes, Nutrient Fluxes, and Agricultural Production. Marcel Dekker, New York, pp 135-172

Douterelo I, Goulder R, Lillie M (2010) Soil microbial community response to land management and depth, related to the degradation of organic matter in English wetlands: Implications for the in situ preservation of archaeological remains. Appl Soil Ecol 44:219-227

Dunger W, Fiedler HJ (1989) Methoden der Bodenbiologie. Gustav Fischer Verlag, Stuttgart

Egli M, Mirabella A, Sartori G, Giaccai D, Zanelli R, Plötze M (2007) Effect of slope aspect on transformation of clay minerals in Alpine soils. Clay Miner 42:375-401

Egli M, Sartori G, Mirabella A, Favilli F, Giaccai D, Delbos E (2009) Effect of north and south exposure on organic matter in high Alpine soils. Geoderma 149:124-136

Egli M, Sartori G, Mirabella A, Giaccai D, Favilli F, Scherrer D, Krebs R, Delbos E (2010a) The influence of weathering and organic matter on heavy metals lability in silicatic, Alpine soils. Sci Total Environ 408:931-946

Egli M, Sartori G, Mirabella A (2010b) The effects of exposure and climate on the weathering of late Pleistocene and Holocene Alpine soils. Geomorphology 114:466-482

Ehrenfeld JG, Ravit B, Elgersma K (2005) Feedback in the plant-soil system. Annual Review of Environment and Resources 30:75–115

Favilli F, Egli M, Brandová D, Ivy-Ochs S, Kubik PW, Cherubini P, Mirabella A, Sartori G, Giaccai D, Haeberli W (2009) Combined use of relative and absolute dating techniques for detecting signals of Alpine landscape evolution during the late Pleistocene and early Holocene. Geomorphology 112:48-66

Fierer N, Schimel JP, Holden PA (2003) Variations in microbial community composition through two soil depth profiles. Soil Biol Biochem 35:167-176

Frey SD, Drijber R, Smith H, Melillo JM (2008) Microbial biomass, functional capacity, and community structure after 12 years of soil warming. Soil Biology and Biochemistry 40:2904–2907

Galvan P, Ponge JF, Chersich S, Zanella A (2008) Humus components and soil biogenic structures in Norway spruce ecosystems. Soil Sci Soc Am J 72:548-557

Graefe U, Beylich A (2006) Humus forms as tool for upscaling soil biodiversity data to landscape level? Mitteilgn Dtsch Bodenkundl Gesellsch 108:6-7

Graefe U, Beylich A (2003) Critical values of soil acidification for annelid species and the decomposer community. Newsl Enchytraeidae 8:51-55

Graefe U, Schmelz RM (1999) Indicator values, strategy types and life forms of terrestrial Enchytraeidae and other microannelids. Newsl Enchytraeidae 6:59-67

Green CT, Scow KM (2000) Analysis of phospholipid fatty acids (PLFA) to characterize microbial communities in aquifers. Hydrogeol J 8:126-141

Hagedorn F, van Hees PAW, Handa IT, Hättenschwiler S (2008) Elevated atmospheric CO2 fuels leaching of old dissolved organic matter at the alpine treeline. Global Biogeochem Cy 22:GB2004

Heuer H, Krsek M, Baker P, Smalla K, Wellington EMH (1997) Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturant gradients. Appl Environ Microbiol 63:3233-3241

Hirsch PR, Mauchline TH, Clark IM (2010) Culture-independent molecular techniques for soil microbial ecology. Soil Biol Biochem 42:878-887

ISO 23611-3 (2007) Soil quality - Sampling of soil invertebrates - Part 3: Sampling and soil extraction of enchytraeids. International Organization for Standardization; ISO 23611-3:2007, Geneva

IUSS Working Group WRB (2006) World Reference Base for Soil Resources 2006. 2nd edition, World Soil Resources Reports No. 103, FAO (Food and Agriculture Organisation of the United Nations), Rome

Jänsch S, Römbke J, Didden W (2005) The use of enchytraeids in ecological soil classification and assessment concepts. Ecotox Environ Safe 62:266–277

Jayasinghe BATD, Parkinson D (2008) Actinomycetes as antagonists of litter decomposer fungi. Appl Soil Ecol 38:109-118

Jenny H (1941) Factors of Soil Formation. McGraw-Hill, New York.

Jenny H (1980) The Soil Resource. Springer, New York

Lalanne A, Bardat J, Lalanne-Amara F, Gautrot T, Ponge JF (2008) Opposite responses of vascular plant and moss communities to changes in humus forms, as expressed by the Humus Index. J Veg Sci 19:645-652

Leidlmair A (1996) Tirol-Atlas. Eine Landeskunde in Karten, Tiroler Landesregierung – Kulturreferat, Alpina Offset, Innsbruck

Mannistö MK, Tiirola M, Haggblom MM (2007) Bacterial communities in Arctic fjelds of Finnish Lapland are stable but highly pH-dependent. FEMS Microbiol Ecol 59:452-465

Margesin R, Jud M, Tscherko D, Schinner F (2009) Microbial communities and activities in alpine and subalpine soils. FEMS Microbiol Ecol 67:208-218

Nakatsu CH (2007) Soil microbial community analysis using denaturing gradient gel electrophoresis. Soil Sci Soc Am J 71:562-571

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G (2003) Microbial diversity and soil functions. Eur J Soil Sci 54: 655-670

Nemergut DR, Costello EK, Meyer AF, Pescador MY, Weintraub MN, Schmidt SK (2005) Structure and function of alpine and arctic soil microbial communities. Res Microbiol 156:775-784

Ning Y, Liu W, An Z (2006) Variation of soil ∂13C values in Xifeng loess-paleosol sequence and its paleoenvironmental implication. Chinese Sci Bull 51:1350-1354

Nübel U, Engelen B, Felske A, Snaidr J, Wieshuber A, Amann RI, Ludwig W, Backhaus H (1996) Sequence heterogeneities of genes encoding 16S rRNAs in Paenibacillus polymyxa detected by temperature gradient gel electrophoresis. J Bacteriol 178:5636-5643

Paterson E, Gebbing T, Abel C, Sim A, Telfer G (2007) Rhizodeposition shapes rhizosphere microbial community structure in organic soil. New Phytol 173:600-610

Paterson E, Osler G, Dawson LA, Gebbing T, Sim A, Ord B (2008) Labile and recalcitrant plant fractions are utilised by distinct microbial communities in soil: Independent of the presence of roots and mycorrhizal fungi. Soil Biol Biochem 40:1103-1113

Ponge JF, Jabiol B, Gégout JC (2011) Geology and climate conditions affect more humus forms than forest canopies at large scale in temperate forests. Geoderma 162:187-195

Ponge JF (2003) Humus forms in terrestrial ecosystems: a framework to biodiversity. Soil Biol Biochem 35:935-945

Risk D, Kellman L, Moroni M (2009) Characterisation of spatial variability and patterns in tree and soil ∂13C at forested sites in eastern Canada. Isot Environ Healt S 45:220-230

Salmon S, Artuso N, Frizzera L, Zampedri R (2008) Relationships between soil fauna communities and humus forms: Response to forest dynamics and solar radiation. Soil Biol Biochem 40:1707-1715.

Sartori G, Mancabelli A (2009) Carta dei suoli del Trentino alla scala 1:250.000. Museo Tridentino di Scienze Naturali, Trento (Italy)

Sboarina C, Cescatti A (2004) Il clima del Trentino – Distribuzione spaziale delle principali variabili climatiche. Report 33, Centro di Ecologia Alpina Monte Bondone, Trento, Italy

Schmelz RM, Collado R (2010) A guide to European terrestrial and freshwater species of Enchytraeidae (Oligochaeta). Soil Organisms 82:1-176

Soil Survey Staff (2010) Keys to Soil Taxonomy, 10th edition. USDA (United States Department of Agriculture), NRCS (National Resources Conservation Service), Washington, DC.

Sørensen T (1948) A method of establishing groups of equal amplitude in a plant sociology based on similarity of species content and its applications to analysis of vegetation on Danish commons. Det. Kong. Danske Vidensk. Selsk Biol Skr 5:1–34

Stark S, Kytöviita MM, Männistö MK, Neumann AB (2008) Soil microbial and microfaunal communities and organic matter quality in reindeer winter and summer ranges in Finnish subarctic mountain birch forests. Appl Soil Ecol 40:456-464

Trigo C, Ball AS (1994) Is the solubilized product from the degradation of lignocellulose by actinomycetes a precursor of humic substances? Microbiology 140:3145-3152

van Vliet PCJ (2000) Enchytraeids. In: Sumner ME (ed) Handbook of Soil Science. CRC Press, Boca Raton, Section C, pp 70-77

White DC, Davis WM, Nickels JS, King JD, Bobbie RJ (1979) Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia 40:51-62

Young IM, Crawford JW (2004) Interactions and self-organisation in the soil-microbe complex. Science 304:1634-1637

Young IM, Blanchart E, Chenu C, Dangerfield M, Fragoso C, Grimaldi M, Ingram J, Monrozier LJ (1998) The interaction of soil biota and soil structure under global change. Glob Change Biol 4:703-712.

Zanella A, Jabiol B, Ponge JF, Sartori G, De Waal R, Van Delft B, Graefe U, Cools N, Katzensteiner K, Hager H, Englisch M, Brethes A, Broll G, Gobatl JM, Brun JJ, Milbert G, Kolb E, Wolf U, Frizzera L, Galvan P, Kolli R, Baritz R, Kemmerse R, Vacca A, Serra G, Banas D, Garlato A, Chersich S, Klimo E, Langohr R (2011a) European Humus Forms Reference Base. http://hal.archives-ouvertes.fr/docs/00/56/17/95/PDF/Humus_Forms_ERB_31_01_2011.pdf

Zanella A, Jabiol B, Ponge JF, Sartori G, De Waal R, Van Delft B, Graefe U, Cools N, Katzensteiner K, Hager H, Englisch M (2011b) A European morpho-functional cassification of humus forms. Geoderma, 10.1016/j.geoderma.2011.05.016

Zogg GP, Zak DR, Ringelberg DB, MacDonald NW, Pregitzer KS, White DC (1997) Compositional and functional shifts in microbial communities due to soil warming. Soil Sci Soc Am J 61:475-481

Table 1 R1

Table 1 Characteristics of the study sites in Val di Fassa – Southern Alps (Italy)

Valley (locality)	Elevation (m asl)	Exposure (°N)	Slope (°)	[a]MAT (°C)	[b]MAP (mm/y)	[c]Thermal sequence	Parent material	Vegetation	Surface age[d]	Land use	Soil type (IUSS Working Group WRB 2006); Soil Taxonomy (Soil Survey Staff 2010)	Humus form (ERB: Zanella et al. 2011a)	Humus form (adapted from Ad-hoc-AG-Boden 2005)
Val di Fassa (Sorda 1)	1620	165	35	4.2	1100	3	Basaltic latite debris	Piceetum	15-18ka	Natural forest (ecological forestry)	Umbric Podzol (Episkeletic) Typic Haplorthod	Hemimoder	F-Mull
Val di Fassa (San Nicolò 4)	1915	195	33	2.5	1200	≤2	Basaltic latite debris	Piceetum	15-18ka	Natural forest (ecological forestry)	Umbric Podzol (Endoskeletic) Typic Haplohumod	Dysmoder	[e]Amphi
Val di Fassa (Sorda 4)	1640	350	36	4.1	1100	2	Basaltic latite debris	Piceetum	15-18ka	Natural forest (ecological forestry)	Umbric Podzol (Endoskeletic) Typic Haplohumod	Eumoder	Typic Moder
Val di Fassa (San Nicolò 2)	1920	300	29	2.5	1200	1	Basaltic latite debris	Piceetum	15-18ka	Natural forest (ecological forestry)	Entic Podzol (Endoskeletic) Typic Haplohumod	Humimor	Mormoder

[a]MAT = mean annual temperature (temperature at a standard altitude of 2 m above ground); [b]MAP = mean annual precipitation (according to Leidlmair 1996)

[c]Thermal sequence considers energy input due to solar radiation (south > north) and MAT. Ranking from the warmest (3) to the coldest (1) site.

[d]Favilli et al. (2009), Egli et al. (2009)

[e]Modified according to Graefe and Beylich (2006)

Table 2 Microannelid species extracted from 4 topsoil profiles in Val di Fassa (Italy) and their ecological classification with respect to soil acidity

	Sorda 1 south	Sorda 4 north	Nicolò 4 south	Nicolò 2 north	Acidity indicator group
Enchytraeidae					
Bryodrilus ehlersi	-	1	-	1	strong
Buchholzia appendiculata	29	59	142	28	slight
Cognettia sphagnetorum	2	56	2	12	strong
Enchytraeus buchholzi	11	1	-	-	slight
Enchytraeus christenseni	2	-	12	-	slight
Enchytraeus norvegicus	-	-	1	-	moderate
Enchytronia oligosetosa	-	1	-	-	slight
Enchytronia parva	-	3	4	-	moderate
Fridericia bisetosa	7	-	3	-	slight
Fridericia bulboides	5	-	10	-	slight
Fridericia christeri	-	-	1	-	slight
Fridericia paroniana	-	-	6	-	slight
Fridericia cf. *stephensoni*	-	2	-	9	moderate
Fridericia waldenstroemi	24	-	1	-	slight
Fridericia sp. juv.	57	6	18	1	slight
Hemifridericia parva	1	-	4	-	slight
Henlea perpusilla	2	-	28	29	slight
Henlea ventriculosa	1	-	-	-	slight
Marionina argentea	2	-	-	-	slight
Marionina brendae	2	-	-	-	slight
Marionina clavata	-	62	-	-	strong
Polychaeta					
Hrabeiella periglandulata	-	48	-	-	moderate
Total of extracted microannelids	145	239	232	80	
Abundance (individuals m^{-2})	73 848	121 722	118 157	40 744	
Number of species	13	10	13	6	
Shannon diversity index	1.81	1.61	1.44	1.38	
Evenness	0.71	0.70	0.56	0.77	
Indicators of strong acidity	1%	50%	1%	16%	
Indicators of moderate acidity	0%	22%	2%	11%	
Indicators of slight acidity	99%	28%	97%	73%	

Table 3 R1

Table 3 PLFA concentrations and $\delta^{13}C$ signature (‰) of the most abundant PLFAs in the studied soil profiles of Val di Fassa (Trentino, Italy). Bacteria used to calculate the fungal to bacteria ratio was defined as the sum of Gram-negative bacteria, Gram-positive bacteria and actinomycete. Microbial biomass was also estimated by the amount of extracted intracellular DNA (iDNA; n=3 ± standard deviation). ~~In order to evidence differences as a function of the sites (Sorda vs. San Nicolò) and especially as a function o exposure (north vs. south), weighted means (wm) (taking the individual soil horizons into account) were calculated for the topsoil and the whole soil profile.~~ The total stocks of the individual components for the different altitudes (Sorda vs. San Nicolò) and exposure (north- vs- south) are also given (in mg/m^2). The total stocks were estimated considering the individual horizon thicknesses, bulk density, concentrations and soil skeleton (see Appendix A and Egli et al. 2009).

Site (exposure)	[a]Horizon	iDNA µg g^{-1} (±s.d.)	Total PLFA µg g^{-1}	Gram-neg. µg g^{-1}	Gram-pos. µg g^{-1}	Actino-mycetes µg g^{-1}	Fungi µg g^{-1}	Fungi / Bacteria	16:00 microbes (generic) $\delta^{13}C$ (‰)	18:1ω7 fungi, G-neg. $\delta^{13}C$ (‰)	18:1ω9 fungi, bacteria $\delta^{13}C$ (‰)	18:2ω6,9 fungi $\delta^{13}C$ (‰)
Sorda 1 (south)	A	24.58 (0.6)	107.4	16.0	11.9	3.73	10.92	0.34	-27.89	-28.73	-26.03	-31.22
	BA	16.85 (1.3)	61.3	11.7	8.0	1.69	6.87	0.32	-26.86	-29.04	-25.31	-31.11
	Bs	4.46 (0.6)	17.7	4.1	2.6	0.47	0.83	0.12	-26.98	-28.88	-26.41	-31.34
Sorda 4 (north)	OE	27.41 (3.0)	296.7	56.3	36.7	4.70	40.47	0.41	-28.52	-29.36	-25.63	-32.31
	AE	40.42 (4.1)	246.7	57.5	38.5	7.32	4.97	0.05	-27.05	-31.7	-26.57	-31.86
	Bs1	23.02 (0.6)	95.7	21.1	17.5	3.27	3.41	0.08	-26.96	-30.29	-27.37	-32.54
	Bs2	10.84 (0.6)	20.2	3.9	4.08	0.63	1.36	0.16	-27.66	-29.03	-30.03	-34.01
San Nicolò 2 (north)	O	24.19 (6.6)	234.7	44.8	29.1	2.84	38.65	0.50	-28.59	-30.82	-26.25	-30.66
	E	25.96 (1.9)	139.0	28.8	16.7	3.99	15.64	0.32	-28.72	-31.84	-28.09	-31.94
	Bhs	17.05 (0.4)	82.0	18.9	18.4	3.04	1.31	0.03	-27.15	-29.89	-27.86	-32.42
	Bs	12.3 (2.6)	4.4	0.98	0.42	0.18		0.00	-26.54	-28.62	-29.23	[b]
San Nicolò 4 (south)	O	22.65 (6.9)	238.1	43.1	38.1	6.21	11.63	0.13	-28.82	-29.34	-27.01	-30.84
	AE	21.89 (1.9)	250.0	41.2	31.6	4.50	33.44	0.43	-28.47	-29.62	-26.7	-30.88
	BA	23.25 (2.0)	107.3	19.3	18.7	2.19	8.74	0.22	-27.16	-29.73	-26.81	-31.28
	Bs1	24.96 (17.9)	30.2	5.42	5.2	0.78	3.19	0.28	-28.28	-30.09	-27.91	-32.53
	Bs2	21.69 (1.9)	11.6	2.49	2.0	0.36		0.00	-26.67	-27.76	-27.76	[b]

Stocks	(mg/m^2)						
Sorda	mean	3878	19455	3843	2736	540	1520
San Nicolò	mean	7165	23587	4508	3641	510	2430
Exposure							
South		6348	18337	3184	2617	466	1779
North		4696	24705	5167	3759	584	2171
Exposure (only topsoil)							
South		2825	15269	2579	2095	380	1590
North		2274	18333	3753	2454	357	1991

[a]Soil depth (cm) of each horizon is reported in Appendix A; [b]PLFA concentration to low for accurate isotope analysis

Table 4 PLFA concentrations and iDNA values standardised to the measured org. C content in the corresponding horizons (see also Appendix A). No org. C data for the O and OE horizons were available.

Locality (exposure)	Horizon	iDNA µg gC^{-1}	Total PLFA µg gC^{-1}	Gram- neg. µg gC^{-1}	Gram- pos. µg gC^{-1}	Actino-mycetes µg gC^{-1}	Fungi µg gC^{-1}
Sorda 1 (south)	A	536	2340	349	259	81	238
	BA	691	2512	480	328	69	282
	Bs	213	847	196	124	22	40
Sorda 4 (north)	OE						
	AE	221	1350	315	211	40	27
	Bs1	268	1115	246	204	38	40
	Bs2	152	283	55	57	9	19
San Nicolò 2 (north)	O						
	E	180	964	200	116	28	108
	Bhs	176	844	195	189	31	13
	Bs	336	120	27	11	5	0
San Nicolò 4 (south)	O						
	AE	283	3234	533	409	58	433
	BA	459	2121	381	370	43	173
	Bs1	953	1153	207	198	30	122
	Bs2	1068	571	123	99	18	0

Table 5 Overview of the principal DGGE results (Fig. 3). Similarities (%) of microbial communities (bacteria, actinomycetes, fungi) as function of ~~thermal conditions (~~exposure (south vs. north), altitude and site (Sorda vs. San Nicolò), and soil depth (horizon) ~~and~~ of the studied soil profiles in the Fassa valley (Southern Italian Alps)

Parameter	Sorda			San Nicolò		
Exposure	*Number of clusters*		Highest similarities	*Number of clusters*		Highest similarities
	South	*North*		*South*	*North*	
Bacteria	one (48%)		Top soils: BA-*S*/OE-*N* (58%)	one (48%)		Bottom-top soils: Bs2-*S*/OH-*N* (60%)
Actinomycetes	one (5%)		Bottom soils: Bs-*S*/Bs1-*N* (65%)	one (5%)		Bottom-top soils: Bs2-*S*/OH-*N* (80%)
Fungi	one (20%)		Top soils: A-*S*/OE-*N* (58%)	one (15%)		Bottom soils: Bs2-*S*/BHs-*N* (48%)

Parameter	Sorda			San Nicolò		
Horizons	*Number of clusters*		Highest similarities	*Number of clusters*		Highest similarities
	South	*North*		*South*	*North*	
Bacteria	one (65%)	three OE/AE/ Bs1,Bs2	Bottom soils-*N*: Bs1/Bs2 (80%)	three O/AE,BA, Bs1/Bs2	two OH,E/ Bhs,Bs	Top soils-*S*: O/AE (80%) Top soils-*N*: E /OH (82%)
Actinomycetes	two (38%) A,BA/Bs	four	Top soils-*S*: A/BA (79%)	three O/AE,BA, Bs1/Bs2	two OH,E,Bhs/ Bs	Top soils-*S*: AE/BA (65%)
Fungi	three (20%) A/BA/Bs	two	Bottom soils-*N*: Bs1/Bs2 (63%)	three O,Bs1/AE, BA/Bs2	three OH,E/Bhs/ Bs	Top soils-*S*: AE/BA (48%) Top soils-*N*: AE/BA (68%)

Parameter	Sorda (South and North) vs. San Nicolò (South and North)
Site	*Number of clusters*
Bacteria	two (38%)
Actinomycetes	two (5%)
Fungi	two (15%)

Sorda (1620-1640 m asl):

South, Sorda 1: A (2-10 cm), BA (20-37 cm), Bs (37-55 cm)

North, Sorda 4: OE (0-5 cm), AE (5-15 cm), Bs1 (15-32 cm), Bs2 (32-50 cm)

San Nicolò (1915-1920 m asl):

South, San Nicolò 4: O (0-4 cm), AE (4-12 cm), BA (12-28 cm), Bs1 (28-50 cm), Bs2 (50-82 cm)

North-facing San Nicolò 2: OH (0-17 cm), E (17-28 cm), Bhs (28-55 cm), Bs (55-90 cm)

Table 6 Comparison of sites on the basis of presence-absence data of microannelid species in a trellis diagram (similarity index according to Sørensen 1948). Highest similarity is between sites with same exposure. The site Sorda north-facing shows the lowest similarity compared to both south-facing sites

	Sorda (south)	Nicolò (south)	Sorda (north)
Nicolò (south)	**0.69**		
Sorda (north)	0.35	0.35	
Nicolò (north)	0.42	0.42	**0.63**

Appendix A Physical-chemical properties of the investigated forest soils located in Val di Fassa. Stable and labile organic matter fractions and C/N ratios of the fine earth (< 2 mm)

Locality (exposure)	Horizon	Depth (cm)	Soil layer thickness (cm)	Bulk density (g/cm^3)	Munsell color (moist)	[a]Sand g/kg	Silt g/kg	Clay g/kg	pH ($CaCl_2$)	pH (H_2O)	org. C g/kg	N g/kg	C/N	[b]Cstab ($g\ kg^{-1}$)	[c]Clab ($g\ kg^{-1}$)
Sorda 1	A	2-10	20	0.87	10YR 2/2	710	170	120	5.0	5.7	45.9	2.20	19.4	8.0	39.7
(south)	BA	20-37	17	1.01	7.5YR 2.5/2	560	230	210	5.3	5.9	24.4	1.40	14.4	6.0	16.9
	Bs	37-55	18	1.18	7.5YR 3/3	720	200	79	5.4	6.0	20.9	1.50	11.0	n.m.	n.m.
Sorda 4	OE	0-5	5	0.61											
(north)	AE	5-15	10	0.61	7.5YR 2.5/1	660	210	130	3.7	4.6	182.7	9.10	19.9	34.8	138.6
	Bs1	15-32	17	0.46	5YR 3/2	530	380	88	4.4	5.8	85.8	3.80	19.7	13.6	72.2
	Bs2	32-50	18	0.92	7.5YR 3/3	590	360	50	4.8	6.0	71.3	3.00	23.7	15.1	43.6
San Nicolò 2	O	0-17	17	0.35											
(north)	E	17-28	11	0.35	7.5YR 3/3	480	220	300	4.0	4.4	144.2	7.20	19.6	26.6	107.1
	Bhs	28-55	27	0.67	2.5YR 2.5/1	720	150	130	4.6	5.3	97.1	4.30	21.6	12.1	84.3
	Bs	55-90	35	1.12	2.5YR 3/2	890	80	30	4.7	5.7	36.6	2.40	13.5	n.m.	n.m.
San Nicolò 4	O	0-4	4	0.35											
(south)	AE	4-12	8	0.77	7.5YR 3/2	590	250	160	4.8	5.4	77.3	5.00	15.4	15.5	57.3
	BA	12-28	16	0.77	7.5YR 2.5/2	610	240	150	5.1	5.6	50.6	3.70	12.0	11.9	36.8
	Bs1	28-50	22	0.86	5YR 3/3	580	260	160	5.3	6.2	26.2	2.00	13.0	9.2	17.0
	Bs2	50-82	32	0.82	7.5YR 4/4	640	260	100	5.5	6.3	20.3	1.50	10.9	7.9	12.4

[a]Size fractions: sand = 2000 - 50 µm, silt = 50 - 2 µm, clay < 2 µm; n.m. = not measured.

[b]Cstab = stable organic carbon (resistant to H_2O_2 treatment); [c]Clab = labile organic carbon (oxidised with H_2O_2)

Figure captions

Fig. 1 Microannelid species composition and abundance (animals 100 cm^{-3} soil) and PLFA concentration (µg g^{-1} dry soil) indicating fungal and bacterial biomass (sum of Gram-negative bacteria, Gram-positive bacteria and actinomycete) as a function of ~~(~~exposure and altitude~~)~~ (thermal conditions) and soil depth in 4 soil profiles (Val di Fassa, Italy): A) Sorda 1 (1620 m asl), south-facing; B) Sorda 4 (1640 m asl north-facing), C) San Nicolò 4 (1915 m asl south-facing), D) San Nicolò 2 (1920 m asl north-facing). Colours indicate the individual species reported in Table 2.

[1)]pH was measured in $CaCl_2$ solution (Appendix A). The pH of first few centimetres of the organic horizon was measured in the field colorimetrically

Fig. 2 Principal component analysis (PCA) of the relative abundance (percentage of total) of PLFAs present in the soils. Connecting lines join horizons of each treatment profile sequentially from the top to the bottom. The shaded ellipses indicate the space occupied by the top two horizons of each profile (Table 3). (South-facing sites: Val Sorda 1, San Nicolò 4; North-facing sites: Val Sorda 4, San Nicolò 2)

Fig. 3 Patterns of bacterial (top), actinomycete (middle) and fungal community (bottom) DGGE fingerprinting and dice-similarity based cluster analysis (UPGAMA) of the studied soil profiles (Val di Fassa, Trento, Italy). Community structures were analysed as a function of ~~thermal conditions (~~exposure (south vs. north), altitude/site (Sorda vs. San Nicolò) and soil depth (horizon) in terms of diversity and vertical distribution. Numbers at the bottom of the DGGE gels indicate the phylotype richness of prominent community members. M, standardised DNA used as intra-gel DGGE marker (Mass RulerTM DNA Ladder Mix; Fermentas). The main results are summarised in Table 4.

South- (S) and north- (N) facing sites of Sorda and San Nicolò (S. Nic.); horizons O, OE, A, AE, BA, Bs (1,2), E, BC (1,2), C; a,b,c replicates of PCR-DGGE

Fig. 4 Acidity indicator diagram of microannelid species assemblages. The position on the triangle shows the relative abundance (percentage of total) of three ecological species groups present in the soils (indicators of slightly acid, moderately acid and strongly acid conditions). The distances visualize the extent to which exposure (south vs. north) has changed the community structure

Figure 1 R1

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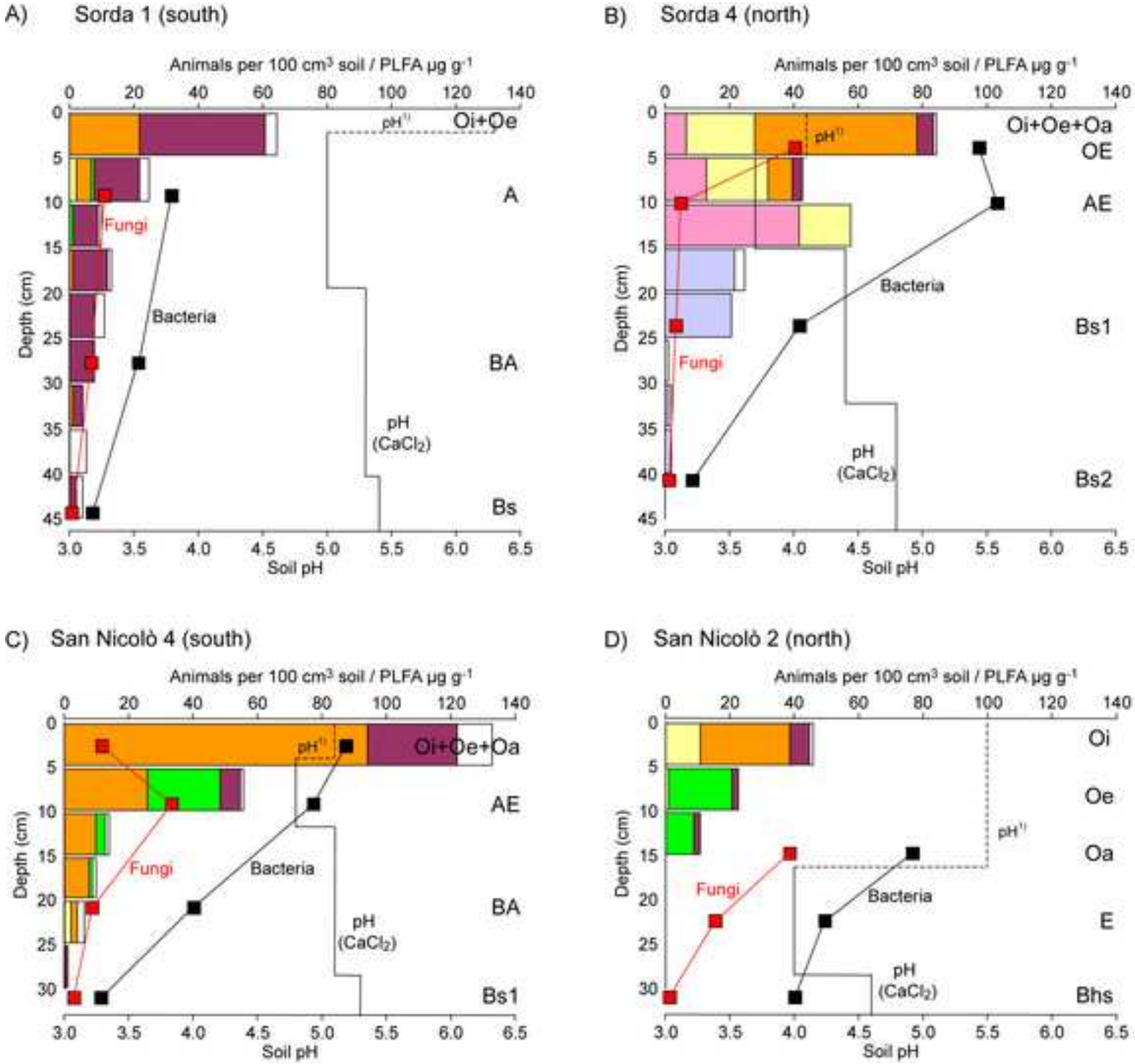

Figure 2 R1

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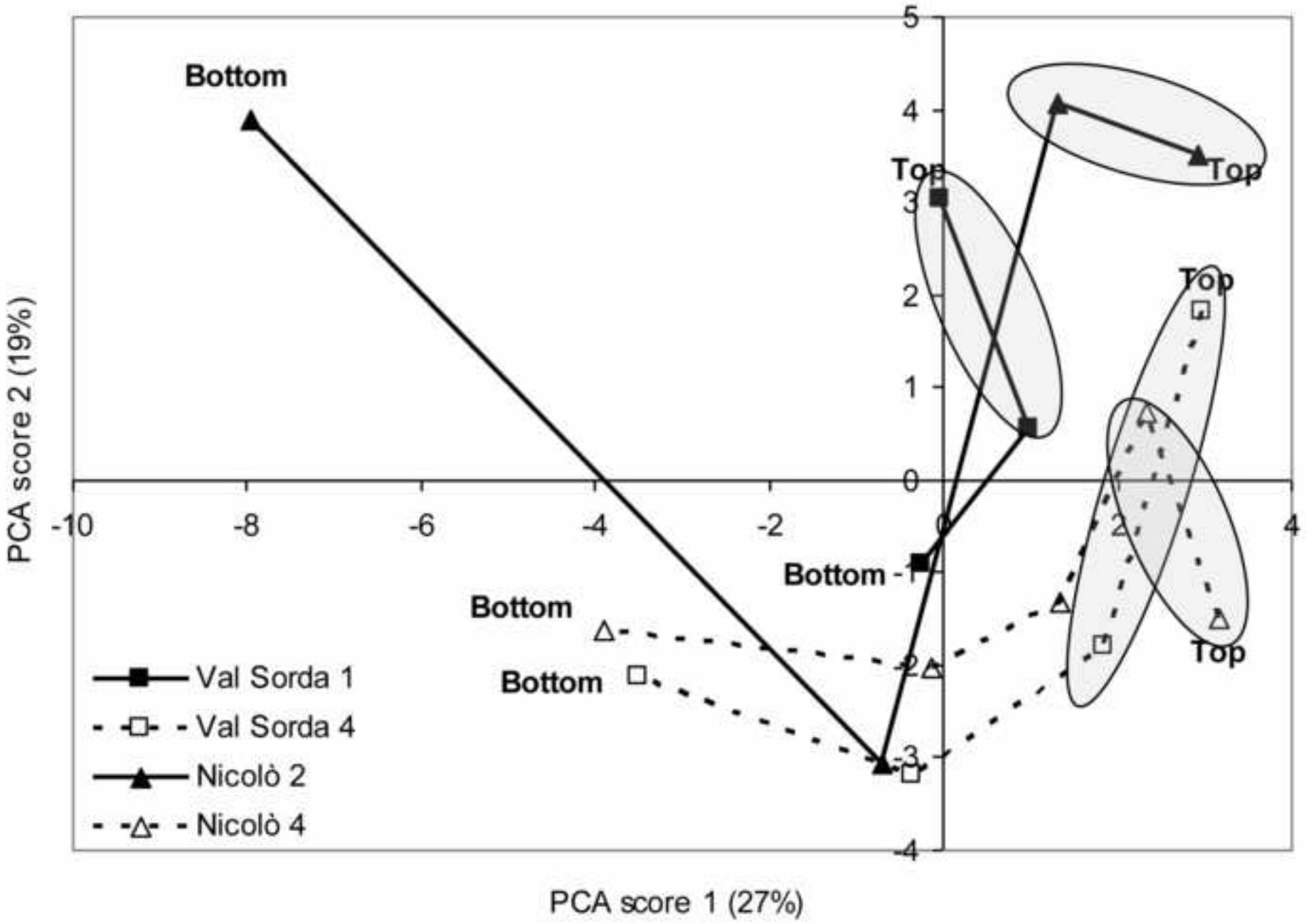

Figure 3 R1

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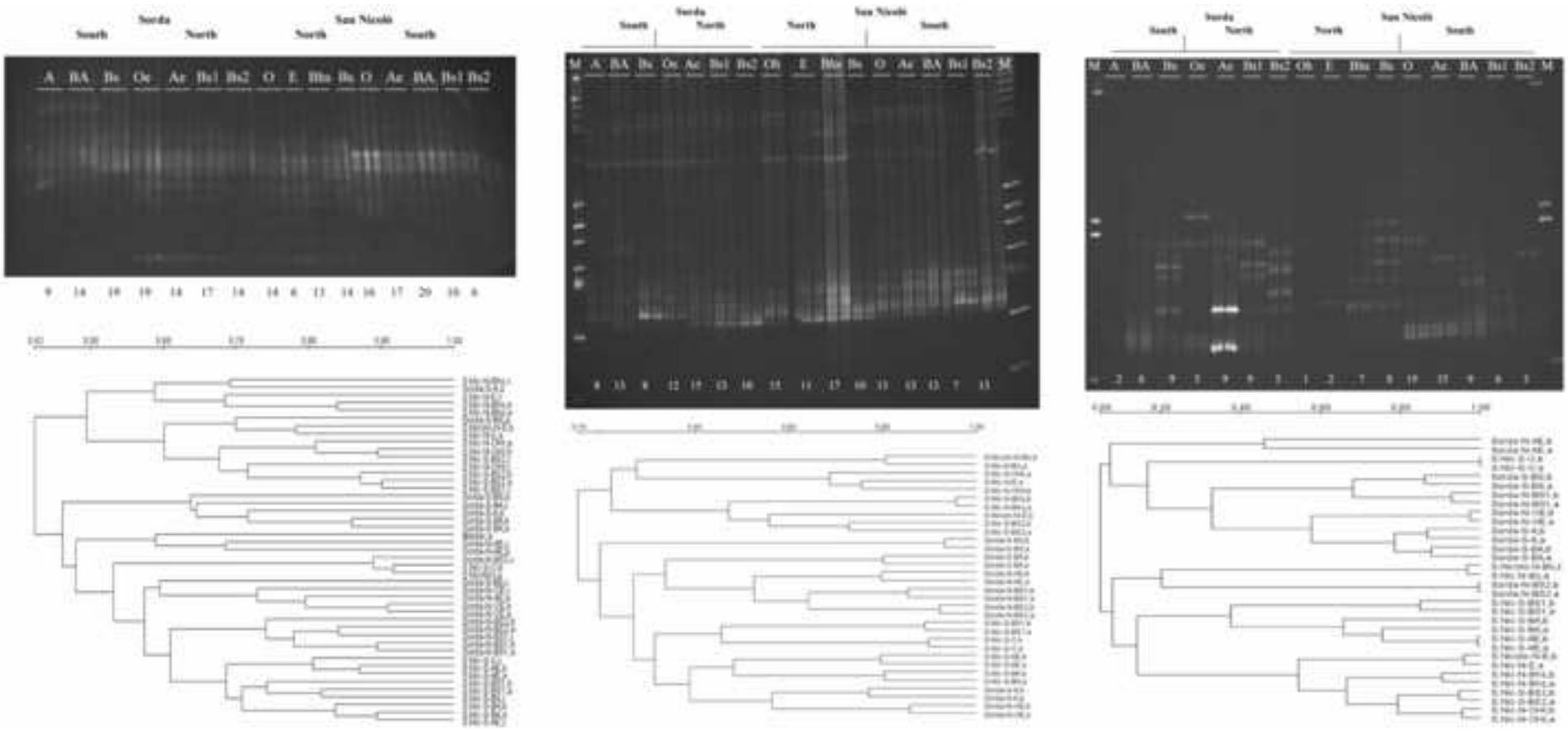

Bacterial community

Fungal community

Actinomycete community

Figure 4 R1

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